Reprinted from the AMERICAN JOURNAL OF VETERINARY RESEARCH, Vol. 49, No. 11, Pages 1928-1931.

Mycotoxicosis caused by aerosolized T-2 toxin administered to female mice

J. D. Thurman, DVM, MS; D. A. Creasia, PhD; R. W. Trotter, DVM

SUMMARY

Thymus, spleen, adrenal glands, and small intestine of female mice exposed to aerosolized T-2 mycotoxin were examined at postexposure hours (PEH) 0.25, 1, 2, 4, 6, 9, 12, and 24. Lymphocyte necrosis was observed at PEH 1 in the thymus, spleen, and lamina propria and Peyer patches of the small intestine. Necrosis of small intestinal crypt epithelial cells was observed at PEH 2, and necrosis of parenchymal cells and increased number of neutrophils were seen in sinusoids of the adrenal cortex at PEH 4. These results indicated that the earliest microscopic evidence of T-2 mycotoxicosis after aerosol exposure was necrosis of lymphocytes in the thymus, spleen, and lamina propria and Peyer patches of the small intestine.

The T-2 toxin is a biologically active fungal metabolite produced by species of the genus *Fusarium*. Cytotoxicosis associated with acute, experimentally induced T-2 mycotoxicosis commonly is observed in immature lymphoid, hematopoietic, and intestinal crypt epithelial cells.[1,a] Epidermal necrosis after topical application has been reported.[2,3] Myocardial changes have been reported in rats and swine after administration of the mycotoxin.[4,5] Recently, we reported adrenal cortical necrosis observed in female, but not male mice, after aerosol exposure.[6]

There are a few reports in the literature on the sequential development of T-2 mycotoxicosis in target tissues; these reports describe lesions in chickens and mice after oral exposure to the toxin.[7,8] Aerosol exposure to T-2 toxin results in peracute to acute toxicosis and recently, has been reported to be 10 times more toxic than systemic administration and at least 20 times more toxic than dermal administration.[9] The objective of the study reported here was to characterize the sequential histologic changes in various target organs of female mice exposed to a sublethal dose of aerosolized T-2 toxin.

Materials and Methods

Mice—Female, 6-week-old Swiss ICR mice, weighing 15 to 20 g each, were obtained from the Walter Reed Army Institute of Research animal breeding colony. They were grouped 6/open-bottom polycarbonate cage and were fed a commercially prepared ration[b] and water ad libitum. Room conditions were maintained at 24 C and 50% relative humidity, and room air was changed 12 times/h. The light cycle was 12 hours. The mice were acclimated (1 week) before the study was begun.

Experimental design—The T-2 mycotoxin in ethanol was administered by 10-minute aerosol exposure to each of 3 groups of 12 mice. Controls consisted of 1 group of 8 mice exposed similarly to nebulized ethanol only and 1 group of 8 mice exposed to air only. Three mice from the T-2/ethanol group, 1 mouse from the ethanol-only group, and 1 mouse from the air-only group were killed (cervical dislocation) at postexposure hours (PEH) 0.25, 1, 2, 4, 6, 9, 12, and 24. To allow for unexpected early deaths, the T-2/ethanol group contained 12 more mice than was required for necropsy. The order of exposure was air only, ethanol only, and T-2 and ethanol.

Chemicals—Purified (> 99%) T-2 mycotoxin was obtained as a white crystalline powder.[c] Purity was confirmed in this laboratory to be > 99% by results of thin-layer chromatography and gas chromatography/mass spectrometry analyses. Synthetic [^{3}H]T-2 mycotoxin in ethanol was obtained (sp act: 11 Ci/mmol; 1.1 μmol/ml).[d]

Aerosol generation—Crystalline T-2 mycotoxin was dissolved in pharmaceutical-grade ethanol to produce a final solution of 25 mg/ml. Fifty microliters of [^{3}H]T-2 mycotoxin in ethanol was added as a tracer. The test aerosol was generated from the T-2 ethanol solution, using a nebulizer.[e] Control mice were exposed to ethanol nebulized in a similar manner.

Aerosol exposure—The dynamically operated aerosol chamber was designed for nose-only exposure to the T-2 mycotoxin aerosol. Basically, exposure was accomplished by placing mice in an open-ended, cone-shaped holder with a hole small enough so that only the nose protruded into a 1.5-L aerosol chamber. The chamber was operated at an exposure rate of 2.2 L/min. The aerosol mass concentration varied between 225 and 275 μg of T-2 toxin/L of air and was determined for each aerosol exposure from a grab sample of the T-2 aerosol taken from the aerosol exposure chamber. The grab samples were obtained by drawing the [^{3}H]T-2 aerosol from the exposure chamber at the rate of 1 L/min for 1 minute across a fiberglass filter[f] held in a standard filter holder.[g] The fiberglass filter then was placed in a standard scintillation vial. The T-2 was extracted overnight from the filter in 1 ml of ethanol; then, 10 ml of scintillation fluid was added, and the [^{3}H] on the filter was quantitated 24 hours later by use of a scintillation counter.[h] The quantity of T-2 on the filter then was calculated on the basis of the known ratio of [^{3}H]T-2 to unlabeled T-2 that was present before nebulization of the ethanol solution of T-2.

Received for publication July 24, 1987.
From the Divisions of Pathology (Thurman, Trotter) and Pathophysiology (Creasia), US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21701-5011. Dr. Thurman's present address is Pathology Associates Inc, NCTR-MC923, PO Box 26, Jefferson, AR 72079.

Address reprint requests to Dr. Trotter.

[a] Hayes MA. *Morphological and toxicological studies on experimental T-2 mycotoxicosis*. PhD thesis, University of Saskatchewan, Saskatoon, 1979.

[b] NIH formula 07, Zeigler Brothers Inc, Gardners, Pa.
[c] Myco-Lab Co, Chesterfield, Mo.
[d] Amersham International, Amersham, UK.
[e] Lovelace nebulizer, Intox Products, Albuquerque, NM.
[f] Intox Products, Albuquerque, NM.
[g] Gelman Scientific Inc, Ann Arbor, Mich.
[h] Model L 55800 Scintillation Counter, Beckman Instruments Inc, Irvine, Calif.

TABLE 1—Extent of necrosis in various organs of female mice exposed to aerosol-administered T-2 mycotoxin

Mouse No.	After exposure	Adrenal gland	Small intestine	Spleen	Thymus
1	15 Min	−	−	−	−
2		−	−	−	−
3		−	−	−	−
4	1 H	−	−	+	+
5		−	−	+	+
6		−	−	+	+
7	2 H	−	+	+ +	+ +
8		−	+	+ +	+ +
9		−	+	+ +	+ +
10	4 H	+	+	+ +	+ +
11		+ +	+	+ +	+
12		+ +	+ +	+	+
13	6 H	+ +	+ + +	+ +	+ +
14		+ +	+ + +	+ +	+ +
15		+ +	+ +	+	+ +
16	9 H	+ + +	+ + +	+ +	+ +
17		+ + +	+ + +	+	+ + +
18		+ + +	+ + +	+	+ + +
19	12 H	+ +	+ +	+	+ + + +
20		+ + +	+ +	+	+ + +
21		+ + +	+ + +	+	+ + + +
22	24 H	+ + +	+ +	+ +	+ + + +
23		+ + +	+ +	+ +	+ + + +
24		+ + +	+ + +	+ +	+ + + +

Extent of necrosis: −, normal; +, minimal; + +, mild; + + +, moderate; + + + +, severe. Grading of adrenal gland is relative to the zona fasciculata. Grading of thymus is relative to the cortex. Grading of the small intestine is relative to the crypt epithelium.

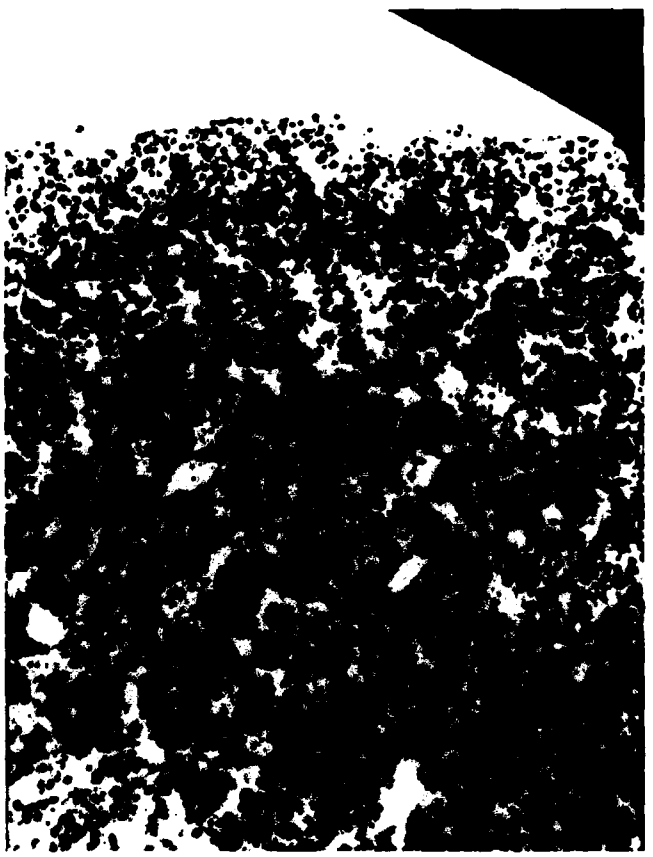

Fig 1—Section of the thymus of a T-2 mycotoxin-exposed female mouse at postexposure hour 24. Notice the extensive pyknosis and karyorrhexis of thymocyte nuclei. H&E stain; × 100.

Fig 2—Section of the spleen of a mycotoxin-exposed mouse at postexposure hour 24. Notice lymphocyte necrosis in the red and white pulp. H&E stain; × 100.

Light microscopic examination—Thymus, spleen, small intestine (ileum), and adrenal glands from each mouse were collected and were fixed in neutral buffered 10% formalin. After dehydration with graded concentrations of ethanol, tissues were placed in an infiltration solution overnight. Tissues were embedded in JB-4 embedding medium, and sections were cut at 3 μm and stained with hematoxylin and eosin (H&E). All tissue sections were examined by light microscopy. A grading system to characterize the extent of necrosis was designed as follows: −, normal; +, minimal; + +, mild; + + +, moderate; + + + +, severe. The criteria for necrosis included any of the following: nuclear pyknosis, karyolysis, karyorrhexis, or lysis of plasma cell membrane. Two veterinary pathologists (JDT, RWT) independently evaluated the 4 target tissues for evidence of necrosis. The final results were obtained with the concurrence of both pathologists.

Results

Female mice exposed to aerosolized T-2 mycotoxin developed necrosis in thymus, spleen, small intestine, and adrenal glands (Table 1). The quantity of necrosis varied with the tissue, but was observed in all tissues within 4 hours of exposure.

Minimal necrosis of lymphocytes was seen in the cortex of the thymus at PEH 1 and increased to a severe grade by PEH 12. At PEH 12 and 24, 80 to 90% of the cortical lymphocytes had pyknotic or karyorrhectic nuclei (Fig 1). Tingible-body macrophages were commonly observed within the cortex. Although the necrosis principally in-

Fig 3—Section of the small intestine of a mycotoxin-exposed mouse at postexposure hour 24. Necrosis of lymphocytes in the lamina propria and of crypt epithelial cells is seen. H&E stain; × 100.

Fig 4—Section of the adrenal gland of a T-2 mycotoxin-exposed female mouse at postexposure hour 24. Notice extensive necrosis of parenchymal cells in the zona fasciculata. Neutrophils and small foci of mineralized debris are seen in the necrotic zone. H&E stain; × 100.

volved the cortex, there was some lympholysis in the medulla.

Minimal necrosis of lymphocytes in the red and white pulp of the spleen was observed at PEH 1. The amount of necrosis was mild by PEH 2 and remained relatively constant through PEH 24 (Fig 2). Some congestion and moderate depletion of lymphocytes were seen in the spleen at PEH 12 and 24. Megakaryocytes in the red pulp remained morphologically unaffected in all mice.

Sections of ileum had minimal necrosis of crypt epithelial cells at PEH 2. The number of necrotic crypt cells increased by PEH 6 and remained fairly constant throughout PEH 24 (Fig 3). A marked decrease in the number of mitotic figures in the crypt epithelium was observed at PEH 2. Almost complete lack of mitotic figures continued until PEH 9, at which time, they began to increase in number. The number of mitoses at PEH 24 did not, however, reach the number seen in control mice. Cytoplasmic vacuolation of enterocytes on or near the villus tips was inconsistently observed between PEH 6 and 12. We observed necrosis of lymphocytes within the lamina propria and Peyer patches at PEH 1. This was a persistent finding during the remaining observation periods. Lacteals occasionally were dilated within the lamina propria of some villi.

Adrenal glands had occasional necrotic parenchymal cells and sparse numbers of neutrophils in sinusoids at PEH 4. These changes were limited to the inner zona fasciculata. At PEH 9, neutrophil concentration and parenchymal cell necrosis and degeneration were increased. The necrosis was characterized by pyknosis, karyorrhexis, karyolysis, cytoplasmic vacuolation, and loss of well-defined parenchymal cell borders and remained constant through PEH 24 (Fig 4). Parenchymal architecture was reasonably well preserved throughout the cortex, with most affected areas maintaining vascular integrity. Multifocal congestion of vascular channels was seen sporadically. Specimens from mice examined at PEH 24 had occasional small foci of mineralization in the zone of parenchymal cell necrosis. Accessory adrenal cortical nodules that occasionally were detected contained zonal necrosis similar to that seen in the cortex.

Air-only and ethanol-only control mice did not have appreciable lesions. Necrosis of individual parenchymal cells in the X zone of the adrenal cortex was sporadically observed in control and toxin-exposed mice; this change is considered normal in maturing mice. In all mice, extramedullary hematopoiesis was seen in the red pulp of the spleen.

Discussion

In this study, necrosis of lymphocytes and epithelial cells in target organs occurred shortly after exposure to T-2 mycotoxin aerosol. Chronologically, cytolysis developed in lymphoid cells before it did in epithelial cells.

Lymphocytes in the cortex of the thymus had morphologic evidence of necrosis starting at PEH 1. These immature T lymphocytes are a rapidly dividing population and often have mitotic indices 10 times that of mature lymph node populations.[10] Lymphocytes in the spleen also had early evidence of necrosis, but this was quantitatively less throughout the 24-hour period than that seen in the thymic cortex. Some necrosis in the red pulp of the spleen undoubtedly involved extramedullary hematopoietic tissue. Lymphocytes in the lamina propria and Peyer's patches of the small intestine also had necrotic changes within 1 hour after exposure to the toxin.

The crypt epithelium of the small intestine was the first nonlymphoid tissue to have cytolytic changes. Chickens given T-2 toxin by crop gavage are reported to develop necrosis in villus tip enterocytes before necrosis develops in crypt epithelium.[9] In contrast, mice of this study developed necrosis in the crypt epithelial cells first (PEH 2) and subsequent vacuolation (degeneration), sporadically in the enterocytes of the villus tips, at PEH 6, 9, and 12. This sporadic vacuolation of villus enterocytes may be related to the decrease in mitotic activity observed in the crypt epithelium. Enterocytes normally migrate from the crypt along the villi; they become senescent and subsequently are sloughed at the villus tip.[11] In this study, the vacuolation of enterocytes corresponded to the period in which crypt mitotic activity was decreased markedly. Prolonging the migration time could amplify the normal degenerative or absorptive changes as the enterocyte approaches the villus tip.

In this study, the necrosis of parenchymal cells of the inner zona fasciculata was detected at PEH 4 and was consistent with our previous observations. In that report,[6] the lesion was observed in female, but not in male, mice after aerosol exposure to the mycotoxin. The specificity of the toxin for causing an adrenal lesion in only one sex of mice appears to be unique. Recent unpublished data from our laboratory indicate that the adrenal lesion also is observed in castrated male mice. Research is currently underway to determine whether exogenous testosterone has a protective effect on the adrenal parenchyma of castrated male mice.

References

1. Committee on protection against trichothecene mycotoxin. *Protection against trichothecene mycotoxins*. Washington, DC: National Academy Press, 1983.
2. Kemppainen BW, Riley RT, Joyave JL, et al. In vitro percutaneous penetration and metabolism of [^{3}H]T-2 toxin: comparison of human, rabbit, guinea pig, and rat. *Toxicon* 1987;25:185–194.
3. Schiefer HB, Hancock DS. Systemic effects of topical application of T-2 toxin in mice. *Toxicol Appl Pharmacol* 1984;76:464–472.
4. Yarom R, More R, Sherman Y, et al. T-2 toxin induced pathology in the hearts of rats. *Br J Exp Pathol* 1983;64:570–577.
5. Pang VF, Adams JH, Beasley VR, et al. Myocardial and pancreatic lesions induced by T-2 toxin, a trichothecene mycotoxin, in swine. *Vet Pathol* 1986;23:310–319.
6. Thurman JD, Creasia DA, Quance JL, et al. Adrenal cortical necrosis caused by T-2 mycotoxicosis in female, but not male, mice. *Am J Vet Res* 1986;47:1122–1124.
7. Hoerr FJ, Carlton WW, Yagen B. Mycotoxicosis caused by a single dose of T-2 toxin or diacetoxyscirpenol in broiler chickens. *Vet Pathol* 1981;18:652–664.
8. Hayes MA, Bellany JEC, Schiefer HB. Subacute toxicity of dietary T-2 toxin in mice: morphological and hematological effects. *Can J Comp Med* 1980;44:203–218.
9. Creasia DA, Thurman JD, Jones LJ III, et al. Acute inhalation toxicity of T-2 mycotoxin in mice. *Fundam Appl Toxicol* 1987;8:230–235.
10. Anderson AO, Anderson ND. Structure and physiology of lymphatic tissues. In: Oppenheim JJ, Rosenstreich DL, Polter M, eds. *Cellular functions in immunity and inflammation*. New York: Elsevier/North-Holland, 1981;32.
11. Moon HW. Intestine. In: Cheville NF, ed. *Cell pathology*. 2nd ed. Ames, Iowa: The Iowa State University Press, 1983;503.